REMARKS

Applicant respectfully requests reconsideration of the present application in view of the foregoing amendments and in view of the reasons that follow. The Advisory Action dated September 11, 2003 states that for Appeal purposes, the proposed amendments will be entered. Applicants have presently amended the claims to include the proposed amendments requested in Amendment filed on June 30, 2003 as requested that the Examiner but applicants request that the Examiner consider the arguments presented and the supporting scientific publications submitted in that amendment and the Supplemental Response filed on July 10, 2003.

Claims 75, 80, 83, 88, 93 and 96 are currently being amended. Claims 101-110 are being added. Claims 75-110 are pending.

This amendment adds and amends claims in this application. A detailed listing of all claims that are, or were, in the application, irrespective of whether the claim(s) remain under examination in the application, is presented, with an appropriate defined status identifier.

Applicants have amended these claims based on previous claims 75 and 88 and the specification on page 42. Claims 75, 80, 83, 88, 90 and 93 have been amended to more clearly define the present invention and claims 101-110 are added to claim specific promoters contained within the recited 5' regulatory region. The Examiner has not made any prior art rejections against the pending claims. The amended claims are intended to more clearly define the claimed invention and is not intended to limit the scope of the invention.

Applicants would like to thank Examiner Wilson for kindly granting a telephonic interview with the undersigned attorney and Dr. Rekha Paleyanda, one of the inventors of the present application, on October 21, 2003, where the claims proposed in the Amendment of June 30, 2003 were discussed.

1. Information Disclosure Statement

Applicants herewith submit an Information Disclosure Statement which lists documents that have been filed during the prosecution of this application that were not previously listed on PTO-1449 or PTO-892 forms, and which were used in support of arguments made in previous responses, i.e., documents A1-A25 provided with the supplemental response filed on July 10, 2003 and A26-A38 were provided with the response filed on March 19, 2001. Copies of these documents have not been provided herewith as

these documents have been previously provided in conjunction with the arguments made in these responses.. Attached hereto are documents A41-A50 that are discussed in the present response.

2. **Claim Objections**

The Examiner objects to a number of claims that he considers to contain typographical errors and to claims in which he wants the article "an" changed to "a." Applicants have amended the claims as requested by the Examiner.

3. **Rejections under 35 U.S.C. 112, first paragraph**

Claims 75-100 are rejected under 35 U.S.C. 112, first paragraph, as allegedly not being adequately described in the specification. The Examiner states that the claims are enabled for a transgenic non-human mammal, whose genome comprises a transgene comprising a nucleic acid sequence encoding a protein operatively linked to a promoter that causes secretion of the protein into the urine of the transgenic mammal, where the protein is expressed and secreted in the urine of the mammal, and are enabled for a method of producing a protein in the urine of the mammal. However, the Examiner states that the claims are not reasonably enabled for using 5' regulatory sequences of the uromodulin, renin, erythropoietin, apolipoprotein E, osteopontin, urinary kallikrein, urinary thrombomodulin, uropontin, nephrocalcin or aquaporin genes to express or secrete exogenous proteins in the urine of transgenic non-human mammals; for using any 3' regulatory sequences to obtain exogenous protein expression in the urine; or expressing an enzyme in the urine of transgenic non-human mammals.

Applicants respectfully disagree with the Examiner's position that the specifically recited 5' regulatory sequences recited in claim 75 and 88 are not reasonably enabled by the present specification. The present application provides sufficient disclosure of regulatory elements and sequences that are useful to express proteins in cells of the urinary tract of the transgenic host with subsequent secretion into the host's urine. See pages 28 to 30, 41 and 42 of the specification.

To reiterate our previous arguments, applicants disclose other suitable regulatory sequences that are useful to drive expression of a protein that it is detectable in urine (see page 29 and 42 of the present application). Specifically on page 28, line 3 to page 29, line 31, the production of proteins in transgenic mammals using promoters and other regulatory sequences of kidney-, bladder- or urinary tract-specific genes is disclosed. Further, Examples

2 and 3 on pages 41 and 42 of the specification disclose gene constructs for expression in the urinary tract and secretion into the urine of the transgenic mammal.

Applicants maintain that the specification provides sufficient support and guidance for a skilled person to prepare the constructs containing a specific 5' regulatory sequence as claimed in claims 75 and 88. In this regard, the independent claims are limited to 5' regulatory sequences from proteins that are known to be associated with urinary tract tissue as disclosed on pages 20-27 of the specification. Indeed, Example 3 (page 42) of the present application states the 5' and 3' sequences of uromodulin, uroplakin,renin, erythropoietin, apolipoprotein E, aquaporin, nephrocalcin, osteopontin-k / uropontin, urinary kallikrein and urinary thrombomodulin genes are used in the present invention.

Applicants argued in the response filed on June 30, 2003, and submitted relevant publications in support of an Appendix A that showed that prior to the December 1, 1997 filing date of the priority document of the present application, scientific publications were available that disclosed promoters from the following genes: uromodulin, renin, erythropoietin, apolipoprotein E, osteoplakin/osteopontin and aquaporin. Additional scientific publications are provided herewith as well as additional arguments to show that the claims that recite specific 5' regulatory genes have been amended. Therefore, in an effort to expedite prosecution, claims 75, 80, 82, 88, 93, and 96 have been amended to recite the 5' and 3' regulatory sequences from these six genes that applicants submit were known and available prior to or at the time that the present application's priority document was filed.
A "patent need not disclose, and preferably omits, what is well known in the art." Hybritech v. Monoclonal Antibodies, Inc., 231 USPQ 81, 94 (Fed. Cir. 1986).

In particular the Examiner has alleged that the specification fails to teach the uromodulin promoter and how it was obtained. In this regard, the Examiner is referred to the publication by Yu *et al.* (1994)(A21) and Pennica *et al.* 1987 (A10), both cited in the present specification, that identifies rat, human and bovine promoters. Thus, applicants maintain that the declaration by Dr. Serguei Soukharev, submitted with the previous response on November 5, 2001, is persuasive in supporting applicants' position that the claims are enabled. These constructs were made using the disclosure of the present invention to produce transgenic mammals that expressed EPO and α1-PI, which is secreted into their urine.

While the specification does not teach "any and all" gene promoters, the regulatory regions of many genes expressed in the urinary tract have been sequenced and are known and available to the skilled artisan. Applicants submit that the skilled artisan is sufficiently

skilled to select, isolate, manipulate and prepare constructs containing appropriate 5' and 3' regulatory elements of the described genes expressed in the urinary tract for use in preparing transgenic mammals that express a specific protein or peptide in cells in the urinary tract with the subsequent secretion into the urine. Both independent claims 75 and 88 require that the protein or peptide be expressed under the control of the recited 5' regulatory sequences which includes a promoter, and then secreted into the urine or detectable in the urine of the mammal, which limits the claims to 5' regulatory sequences that result in the expression of the protein or peptide in the cells of the transgenic mammal with the subsequent secretion of the protein or peptide into the urine of the mammal.

In support of this position, applicants submit that all the promoters currently claimed were known and available at the time of application, in Dec 1, 1997. Applicants provide a case by case discussion of each of the claimed 5' regulatory regions that contain promoters that drive the expression of proteins to which genes encoding these proteins are operably linked resulting in expression and secretion into the urine. These promoters are then inserted into constructs to produce transgenic non-human mammals according to the present invention to express proteins in the urine. The evidence provided in Dr. Soukharev's declaration and in the post-filing date published papers support applicants' position that these promoters are useful to carryout the present invention.

For example, uromodulin (UROM/THP) is the most abundant protein in urine. The gene for UROM, its 5' promoter sequence, and its mRNA were known and available as early as 1987(Pennica et al) (A10), see the specification, page 20, line 35 to page 21, line 35. The rat and bovine genes were cloned & promoters identified by Yu et al, 1994 (21). Comparisons with the human gene and about 600 bp promoter showed high level of sequence similarity.

Additionally, once the genes containing these promoters have been located, those skilled in the art can isolate the upstream sequences containing the promoter region, without undue experimentation. Support for this is present throughout the specification, see for example, page 19, line 22 to page 20, line 34, in particular for references detailing procedures that are available to one skilled in the art; see general cloning manuals, such as Maniatis *et al.* 1982, Sambrook *et al.* 1989, and Puhler A, Ed, 1993 (copies not provided). Also, Example 2, on page 41 of the specification describes one of these methods, which was used to isolate the human Uromodulin promoter in applicants' laboratory. In combination with the above references, there is ample information in the specification for one skilled in the art to isolate and assay the Uromodulin promoter, as well as other urinary tract-specific (UTS)

promoters. One way to determine or obtain the promoter is to sequence it, to compare it to published or predicted sequences, and to perform DNA binding assays. Additional functional characterization of the promoter can include generating recombinant DNA constructs and to express it *in vitro*, or *in vivo*, in a transgenic mouse, as supported on page 19, line 22 to page 20, line 4, page 32, line 10 to page 34, line 31, and in the 132 declaration by Dr. Soukharev submitted with the response filed on November 5, 2001, followed by peer-reviewed publications (Zbikowska *et al*, A22 and A23 in the PTO-1449, previously submitted).

Further in this regard, in applicants' 132 declaration by co-worker, Serguei Soukharev, the process of generating transgene constructs containing the UROM promoter isolated in Dr. Lubon's (since-deceased) laboratory at the time of filing is described. Also taught is the generation of transgenic mice using 3.9 kb of the human UROM promoter to target protein expression to the urinary tract. This data showing kidney-specific targeting of (1) the lac Z gene encoding the ß-galactosidase enzyme, (2) the cDNA for hEPO hormone and the protease inhibitor α1-antitrypsin, has since been published as Zbikowska et al, *et al*, A22 and A23, *supra*. ß-galactosidase protein was expressed and demonstrated in kidney using histochemistry, while rhEPO was assayed by ELISA and western blotting of urine (Zbikowska et al, A22). α1-antitrypsin was assayed by ELISA and western blotting of urine, followed by N-terminal sequencing and two bioassays (Zbikowska et al, A23).

Applicants chose to use the human uromodulin promoter, however, they could have instead/also used the mouse or bovine promoter that were identified as early as 1994, Yu *et al.* (A21), to achieve similar results. The high level of inter-species promoter sequence conservation shown by Yu et al, 1994, was confirmed later by Zhu *et al.*, 2002 (A24)and Kim *et al.*, 2003 (A6). Evidence that the UROM promoters can be used to target protein expression to the kidney, with secretion of protein into urine, as disclosed and claimed in the present invention, also was confirmed by other post-application publications, that have used the mouse and bovine promoters to generate transgenic mice. Zhu *et al.* 2002 (A24), identified a 3 kb mouse promoter and used it to generate transgenic mice. Transgenic green fluorescent protein was identified in kidney by fluorescence microscopy, while stable levels of human growth hormone in urine of all 3 lines of transgenic mice was determined by radioimmunoassay (Zhu et al, 2003, A25). Kim et al., 2003 (A6) isolated & sequenced a 3.9 kb bovine promoter and targeted ß-galactosidase protein to kidney, demonstrated by histochemistry. These post-published papers show that it was within the skill of the artisan to use these promoters that were enabled as of the filing date of the present application, and to employ them for targeted protein expression. The submitted publications, such as Yu et al.,

Pennica et al. (both referenced in the present application) and Pook et al. (A11), show that uromodulin promoters were identified and isolated prior to the filing date, and thus support that claims reciting a uromodulin promoter are enabled and the specification provides an adequate written description to isolate a uromodulin promoter.

In the invention, applicants teach the use of gene promoters of proteins known to be directly secreted into urine, such as uromodulin, aquaporin, uropontin/osteopontin. Secondly, applicants also teach the use of the urinary tract (UT)-specific regulatory sequencs present in other genes that are also known to be expressed in the UT, and whose protein products are secreted into urine, such as EPO, renin and apolipoprotein E. Dissection of tissue-specific regulatory elements present in promoters of these genes was known in the art, and either endogenous genes or reporter genes, were used for expression.

Applicants provide further evidence that the other recited 5' regulatory regions comprising promoters were known prior the filing date of the present application and therefore persons skilled in the art would have been able to identify and isolate the promoters from the following proteins:

Aquaporin: This group of proteins is expressed in the urinary tract and secreted into urine in the normal state. For eg. AQP-2 has been detected in urine by RIA (Rai et al, Sep 1997, A12) and immunoblotting (Wen et al, 1999, A49). The AQP2 protein is a present in the collecting ducts in kidney. The human promoter was isolated by Hozawa et al. in 1996 (A44), rat and mouse AQP2 gene & promoters by Rai et al, 1997 (A12). All publications but Wen were published prior to the filing date of the present application. Post-application, transgenic mice were generated using the human AQP2 promoter to target the gene for the Cre recombinase enzyme to the collecting tubules in kidney, Nelson et al, 1998 (A46), Stricklett et al. 1999 (A18). The mouse AQP-2 promoter also achieved renal expression of green flourescent protein in transgenic mice, Zharkhik et al, 2002 (A50), as detected by fluorescence microscopy. These authors did not test for urinary presence of protein, as their main goal was in achieving cell-specific targeting. Applicants recite these promoters as useful for expression proteins for expression in the urine.

AQP CD gene and promoter were isolated in 1994, Uchida et al. (A19), see specification page 24, line 21 to page 25, line 3; AQP-3 isolated in 1995, Inase et al. (A4) (and its promoter used to express luciferase in cells. Post-application, human AQP-1 promoter was isolated by Umenishi et al, 1998 (A20). Thus these AQP promoters were known and available and it is within the skill of the artisan to identify and isolate them for UT-specific expression according to the present invention.

Uropontin/Osteopontin: Uropontin was isolated from urine by Shiraga et al., 1992 (A15) and identified by comparison to osteopontin. Also known as osteopontin or bone sialoprotein I, it has been detected in urine of patients with stones. The bovine cDNA was cloned and mRNA isolated in 1991, by Kerr et al . The cDNA of hOPN was cloned in 1992, by Kohri et al.(A45). The human OPN gene and its 2.3 kb promoter and 5' regions was cloned in 1994, by Hijiya et al. (A43). The mouse promoter was also cloned by them. Transgenic mice have been created using only 910 bp of OPN promoter, however sequences targeting kidney expression were absent from this construct, Sakuma et al., 2003 (A47). Its mRNA was localized in kidney, by Jiang et al., 1998 (A5) and protein detected by immunohistochemistry. The protein is expressed in renal tubules and collecting ducts, and has been identified by ELISA and Western blotting of urine.

Erythropoietin (EPO): EPO is naturally secreted into urine. The liver is the major site of production, but the kidney synthesizes EPO too. Transgenic mice were created using EPO promoter sequences in 1991 (A13) & 1994 (A14) by Semenza et al. The EPO promoter was used to drive lac Z gene expression to kidney by Haider et al., 1996 (A3). A kidney-specific regulatory region was identified in the 1.2 kb 3' flanking sequences. In April 1997, ß-galactosidase enzyme activity was demonstrated in PCT cells by electron microscopy. The current invention teaches that these hormones and enzymes would be secreted into urine. Most of the studies conducted on EPO were done to define its role in hematopoiesis and anemia therapy, thus plasma effects of EPO were studied, and not urinary levels of protein.

Apolipoprotein E: Human, mouse and rat genes and promoters were known and available in 1990, Simonet et al. (A17); see the specification, page 28, line 3 to page 29, line 6. Smith et al, 1990 (A48), showed high level expression in kidney of 3 lines of mice. Simonet et al, show an elegant dissection of the regulatory elements in the promoter, showing that a downstream element 3' of ApoE gene was needed for liver-specific expression. In the absence of this element, the promoter targeted high-level expression to the kidneys, and protein was demonstrated by immunohistochemistry. Thus, for purposes of this invention, applicants teach the 5 kb of 5' flanking sequences and 1.5 kb 3' sequences can be used to efficiently target expression of proteins to the kidney, and that the protein would be secreted into urine. Most of the studies conducted on ApoE were done to define its role in lipoprotein and cholesterol metabolism, thus plasma levels of Apo E were studied, and not urinary.

Renin: The renin promoter & gene were use to generate transgenic mice by Mullins et al, in 1988 (A9). Tissue-specific expression of SV40-T antigen was obtained using the renin promoter in mice, by Sigmund et al., 1990 (A16). Further dissection of regulatory

elements in renin promoter showed that more than 5.7 kb of the human renin promoter were needed to result in specific expression of beta-galactosidase in the kidney, Germain et al., 2001 (A41). Applicants submit that these promoter regions were known and identified as of the filing date of the present invention for use in the present invention to target protein secretion into the kidney. Most of the studies conducted on renin were done to define its role in hypertension, thus presence and level of protein secreted into urine were not determined.

In regard to the 3' regulatory sequences result in the expression of the exogenous gene with subsequent expression into the urine, these sequences are contained in constructs that also contain the recited 5' regulatory sequences of claims 75 and 88 that result in the expression of the protein or peptide in the cells of the urinary tract of the mammals with the subsequent secretion into the urine of the non-human transgenic mammals. A skilled person is capable of selecting and testing appropriate, functional 3' regulatory sequences without undue experimentation from known genes that encode proteins that are associated with the urinary tract of mammals. In view of the above arguments and Dr. Soukharev's declaration, it is requested that this rejection be withdrawn.

In view of the above amendments, arguments and supportive publications showing that the promoters were known and isolatable prior to the filing date of the present invention and by the 132 declaration and post-published papers that support the use of these promoters according to the present disclosure to obtain targeted expression in transgenic animals, it is requested that this rejection be withdrawn.

3. Rejection under 35 U.S.C. § 112, second paragraph

All of the claims are rejected as allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter. A review of the Office Action dated January 8, 2002 sets forth the Examiner's basis for this rejection for improper Markush groups in claims 75, 80, 83, 88, 93 and 96. Applicants have amended these claims to clearly set forth that the Markush group consists of regulatory sequences of the recited genes. It is believed that these amendments to these claims clarify the invention, and it is requested that this rejection be withdrawn.

Conclusion

Applicants submit that this application is in condition for allowance, and they solicit an early indication to that effect. Should the Examiner believe that further discussion of any remaining issues would advance the prosecution, a telephone call to the undersigned, at the telephone number listed below, is courteously invited.

The Commissioner is hereby authorized to charge any additional fees which may be required regarding this application under 37 C.F.R. §§ 1.16-1.17, or credit any overpayment, to Deposit Account No. 19-0741. Should no proper payment be enclosed herewith, as by a check being in the wrong amount, unsigned, post-dated, otherwise improper or informal or even entirely missing, the Commissioner is authorized to charge the unpaid amount to Deposit Account No. 19-0741. If any extensions of time are needed for timely acceptance of papers submitted herewith, Applicant hereby petitions for such extension under 37 C.F.R. §1.136 and authorizes payment of any such extensions fees to Deposit Account No. 19-0741.

Respectfully submitted,

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